California Crop Improvement Association
Disease Inspection Program
Procedures

Introduction

The California Crop Improvement Association (CCIA) Disease Inspection Program is a quality assurance program for the seed industry employing the best field and laboratory diagnostic methods for detection of seed-borne pathogens. This program may be used as part of a company quality control plan in the production of specific pathogen-free vegetable and field seed. Phytosanitary inspections must be made independently of this program.

Pathogen: *Acidovorax avenae* subsp. c*itrulli*

Hosts: Muskmelon, Cantaloupe, Honeydew, Casaba and Watermelon.

1. Definitions

1.1 Qualified Seed Company Inspector:

An individual from a seed company (i.e. quality assurance personnel, plant pathologist or field representative) that has attended and completed one of the CCIA's bacterial fruit blotch training workshops **[**see Bacterial Fruit Blotch Annual Workshop Outline (Page 11)]. Each qualified seed inspector will be issued a certificate of completion and qualification. This person should be familiar with, and agree to follow the procedures outlined in this manual.

1.2 Accredited laboratory:

An independent seed testing laboratory or seed company laboratory approved by the CCIA which uses diagnostic techniques as indicated by the ASTA Ad Hoc Watermelon Fruit Blotch Committee on September 6, 1994 (Recommendation for Detection of the Watermelon Fruit Blotch Pathogen on Watermelon Seeds) and operates under the supervision of a trained plant pathologist. This laboratory may be audited by the CCIA at any time to assure such techniques are being employed and good laboratory operating procedures are being followed [See Laboratory Accreditation Procedures Outline (Page 12)].

2. Application

2.1 Application forms for the Disease Inspection Program are available from the CCIA office (see attachment 2.1).

2.2 Seed companies growing seed using the procedures of the Disease Inspection Program must complete one application per field. If more than five fields are in close proximity, the applicant may fill out one application indicating that it is for multiple fields and include the total area (acreage or hectares) to be inspected. This single application must include the necessary information that describes all individual fields (see attachment 2.2).

2.3 Completed application forms must be sent to the CCIA no later than when seeds have been planted either in the greenhouse or in the field**.**

3. Schedule for Inspections

A minimum of two greenhouse or field inspections will be performed during the growing season. Additional inspections may be performed as deemed necessary to verify pathogens. These inspections may be done by CCIA personnel and/or a Qualified Seed Company Inspector. <u>All fields should be inspected by a CCIA inspector or designated agent at least once</u>, normally at the mature fruit stage. A Qualified Seed Company Inspector may be authorized to make both inspections.

3.1 Greenhouse Transplants

3.1.1 When seeds are planted in U.S. greenhouses for transplants, the applicant must provide CCIA with the seeding and approximate transplant date. A seedling inspection must be made when the first true leaf has expanded (i.e. 2-3 weeks after planting). This mandatory inspection must be performed by a Qualified Seed Company Inspector or by a CCIA inspector.

3.1.2 If suspicious plants are found (water-soaked spots on tissues, necrotic, tan lesions, collapsed plants) proceed as indicated in Section 4). Tissue sampling, documentation (pictures if possible) and analysis will be required. The Hydros Inc., AgriCheck System for Watermelon Fruit Blotch should be utilized immediately (see attached test protocol). If results are positive, confirmation of pathogen presence is necessary by lab isolation and identification of the bacteria, or by seedling growout. Results of this analysis must be submitted to CCIA. Methods used for the analysis must be noted in this report.

3.1.3 If the greenhouse is located abroad, <u>a Qualified Seed Company Inspector must perform this inspection.</u>

3.1.4 If bacterial fruit blotch is found in a greenhouse complex the CCIA must be contacted immediately to determine certification status. Seedling lots will be considered on an individual basis for certification purposes.

3.1.5 If this inspection is done by a Qualified Seed Company Inspector, an inspection report must be submitted to the CCIA, even if no suspicious plants were found (see attachment

3.1.6). This report must include all laboratory results if suspicious plants were sent for analysis.

3.2 Direct Seeded Fields

3.2.1 An inspection of direct seeded seedlings must be made during the first or second true leaf stage. This mandatory inspection must be performed by a Qualified Seed Company Inspector or by a CCIA inspector. Care should be taken to note plants showing symptoms of wilting, or collapsing tissue with watery lesions.

3.2.2 If suspicious plants are found (water-soaked spots on tissues, necrotic, tan lesions, collapsed plants) proceed as indicated in Section 4. Tissue sampling, documentation (pictures if possible) and analysis will be required. The Hydros Inc., AgriCheck System for Watermelon Fruit Blotch should be utilized immediately (see attached test protocol). If results are positive, confirmation of pathogen presence is necessary by lab isolation and identification of the bacteria, or by seedling growout. Results of this analysis must be submitted to CCIA. Methods used for the analysis must be noted in this report.

3.2.3 For fields smaller than 1 acre, inspections should be done by walking every two rows; for fields between 1 to 5 acres, every four rows should be walked, and in fields larger than 5 acres, every 8 rows should be walked.

3.2.4 If this inspection is done by a Qualified Seed Company Inspector, an inspection report must be submitted to the CCIA, even if no suspicious plants were found (see attachment 3.1.6). This report must include all laboratory results if suspicious plants were sent for analysis.

3.3. Mature fruits

3.3.1 <u>This inspection is made by the CCIA or designated agents</u> at the mature fruit stage (1-2 weeks prior to harvest). If the CCIA inspectors or agents cannot inspect a field at the optimum mature fruit stage (due to early or late plantings), a Qualified Seed Company Inspector may be authorized to assist in completing the inspection.

3.3.2 For fields smaller than 1 acre, inspections should be made by walking every two rows; for fields between 1 to 5 acres, every four rows should be walked, and in fields larger than 5 acres, every eight rows should be walked.

3.3.3 If suspicious tissues are found (water-soaked spots on fruits, necrotic, tan lesions, fruit cracking, discoloration or exudate) proceed as indicated in Section 4. Tissue sampling, documentation (pictures, if possible) and laboratory analysis by an accredited lab will be required. The Hydros Inc., AgriCheck System for Watermelon Fruit Blotch should be utilized immediately (see attached test protocol). If results are positive, confirmation of

pathogen presence is necessary by lab isolation and identification of the bacteria, or by seedling growout. Results of this analysis must be submitted to CCIA.

3.3.4 If this inspection is done by a Qualified Seed Company Inspector, an inspection report must be submitted to the CCIA, even if no suspicious plants were found (see attachment 3.1.2). This report must include all laboratory results if suspicious plants were sent for analysis.

4. Sampling

4.1 Seedlings

If suspicious symptoms are observed in the greenhouse or field (water-soaked spots on tissues, necrotic, tan lesions, collapsed plants), tissues must be sampled immediately using the Hydros Inc., AgriCheck System for Watermelon Fruit Blotch. If results are positive, tissue should be properly handled, preserved and submitted to an accredited laboratory as soon as possible. Follow procedures described in the Procedure for Handling and Shipping of Disease Tissue (Page 9). Contact the CCIA for a list of Accredited Laboratories for bacterial fruit blotch analysis. If possible, take pictures of the sample in the greenhouse or field before shipping. A copy of the laboratory analysis submission form and the laboratory results should be sent to the CCIA as soon as they are available.

4.2 Mature fruits

If suspicious symptoms are observed on fruits (watery spots, fruit cracking, discoloration or exudate), take samples as indicated (Page 9).

4.3 Seed

Prior to certification tagging, all seedlots must be sampled using AOSA, AOSCA or ISTA seed sampling procedures by trained personnel. Seed must be tested for pathogen presence (30,000 seedlings minimum for seed lots larger than 300,000 seeds, or 10% by count, seeds from smaller seed lots) in an Accredited Laboratory. Tests must be performed according to established procedures (ASTA Ad Hoc Watermelon Fruit Blotch Committee recommendations for detection of the Watermelon Fruit Blotch Pathogen on Watermelon Seeds). Copies of results must be forwarded to the CCIA prior to tagging.

4.4 The applicant is responsible for maintaining a 35,000 PLS or 10% by seed count, archive sample for BFB testing for the duration of the marketing of that seedlot.

5. Final Report

5.1 Disease Inspection Program Report

5.1.1 Upon the completion of the mature fruit field inspections and receipt of all company field and lab reports, the CCIA will issue a "Disease Inspection Program Report." This report will include all information to identify the inspected seed lot. This report will use the field descriptors provided by the seed company which may include: Field Production Order (FPO) number, cross number, material number, name of variety, area (acreage/hectares) inspected, transplant date, and grower name. No "Disease Inspection Program Report" will be issued before receipt of all required company field and lab reports.

5.1.2 The Disease Inspection Program Report will be sent to the appropriate designated company representative within three weeks after receipt of all company field inspections and lab reports. Upon request by the seed company quality assurance representative, additional copies can be provided to other company personnel, such as field representative, inspectors or district managers.

6. Auditing of Seed Company Records.

The CCIA may perform an audit at any time of all records concerning seed lots that are entered into the Disease Inspection Program. If documentation and record keeping are inadequate, the company will be required to immediately address any deficiencies (see Non-Compliance Procedures section). Failure to implement these procedures may result in termination of the program for that company.

7. Labeling of seed containers.

After each seed lot is tested and no evidence of *Acidovorax avenae* subsp. *citrulli* is detected, the seed is eligible to be tagged with Disease Inspected Labels. These series numbered Disease Inspected Labels should be requested from the CCIA to use on each seed container. Each container should be labeled. Requests to print the Disease Inspected Label on company labels will be considered on an individual basis.

8. Certificates for seed to be shipped to Mexico.

Based on an agreement with the Departamento de Sanidad Vegetal in Mexico, seed can be shipped into Mexico with a Disease Inspection Program Certificate in lieu of a Phytosanitary Certificate for bacterial fruit blotch. The CCIA will send a signed and numbered Disease Inspected Program Certificate to the seed company for seed lots that have passed all criteria in the program. The seed company must complete each Disease Inspection Program Certificate and include the series numbers of the Disease Inspection Labels used in the packaging of each seed lot. The white (original) sheet of this certificate

must accompany the seed shipment, the seed company keeps the yellow copy, and the pink copy should be sent to the CCIA (Attachment E).

9. Non-Compliance Procedures

9.1 If during an audit or at other times it is found that field inspections, sampling, testing and appropriate record keeping procedures are not being performed as outlined, documentation of the process required to overcome any deficiencies must be made and presented to the CCIA. Failure to address these deficiencies may lead to suspension or termination of the program.

9.2 Suspension or termination of the program will occur if:

a) False or misleading information has been deliberately provided in field inspection, laboratory reports or record keeping.

b) Qualified Seed Company or Accredited Laboratory Personnel do not follow correct procedures during inspections or seed health testing.

10. CCIA Responsibility

All technical, genetic and production information will be handled in a strictly confidential manner. CCIA will provide all pertinent program records in support of customers in possible legal challenges. If requested, CCIA will also outline and summarize the program to demonstrate its effectiveness as a quality control plan for the production of specific pathogen-free agricultural seed.

Procedures for
handling and shipping of diseased (suspicious) tissue.

1. If the sample is collected in the U.S., proceed in the following manner:

a) Seedling sample from greenhouse or field.

* Take pictures if possible.
* Sample suspected seedlings by clipping off at the soil line (avoid carrying soil or planting medium with samples.
* Blot off soil and free moisture from sample with clean towel.
* Wrap in a clean paper towel and place inside a sealable plastic bag.
* Label sample inside and out.
* Expel air and close

b) Leaf sample from field.

* Take pictures if possible.
* Remove suspected leaves.
* Blot off soil and free moisture from sample with clean towel.
* Label sample inside and outside the bag with appropriate sample information.
* Wrap and gently flatten leaves in a clean paper towel and place inside a sealable plastic bag.
* Expel air and close.

c) Fruit

* Take pictures if possible.
* Cut off the suspect lesion with clean knife, going only as deep into the flesh as necessary to sample the infected tissue. Avoid lesions that are showing signs of soft rot and excessive decay, but if it is necessary to sample such lesions, take the sample from the area of firm tissue along the lesion margin.
* Clean the knife with rubbing alcohol or other disinfectant between samples.
* Blot off soil and free moisture from sample with clean towel.
* Wrap in a clean paper towel and place inside a sealable plastic bag.

* Label sample inside and outside the bag with appropriate sample information.
* Expel air and close.

NOTE:

- Do not place wet towels or extra moisture in the bag with the sample as this can promote decay.
- Refrigerate samples immediately (ca. 4C) and keep cool until shipped. If refrigerator is not available, allow samples to air dry, then place it in a plastic, paper or glassine bag and store in a cool, dry shaded location.
- Ship samples by next day service to an accredited laboratory for analysis.
- Keep samples from different locations and varieties separate at all times. Do not mix samples from different sources together in a bag.
- Label samples both inside and outside the bag with appropriate sample information.

2. If the sample is collected from areas outside of the U.S., proceed in the following manner:

Use the same procedures as indicated for samples collected in the U.S. However, if you want to send this material to a lab in the U.S. you may require special permits for customs authorities that will allow you to bring or send these samples into the U.S.

Outline of

Bacterial Fruit Blotch Annual Workshop

1. Bacterial Fruit Blotch Workshop (required)

A. Classroom (slide show)

Basic information about pathogen (*Acidovorax avenae* subsp *citrulli*) and disease (Bacterial Fruit Blotch).

B. Greenhouse or Laboratory

Symptoms on seedlings, visual diagnosis and use of Affini Tip kit (seedlings and fruits).

C. Field observation

Visit a field plot and observe fruit symptoms on different watermelon varieties (different "skin" types).

2. Gummy Stem Blight (Optional)

A. Classroom (slide show).

Basic information about pathogen and disease

B. Greenhouse or Laboratory

Symptoms on seedlings, visual diagnosis and lab procedures.

C. Field observation

Visit a field plot and observe symptoms on different tissues of watermelon plants.

Laboratory Accreditation Requirements

Applicant shall:

a) Employ good laboratory practices including, but not limited to, aseptic technique, identification of contamination and containment procedures, sterilization and desinfestation of microbiological materials.

b) Have a quality assurance program and manual.

c) Assure that all equipment is properly maintained and calibrated.

d) Make staff aware of job duties and the extent and limitations of their responsibilities. Have documentation of appropriate education, knowledge and experience necessary to perform tests.

e) Have at least one university-trained pathologist supervising seed health tests.

f) If possible, assure seed samples have been drawn according to AOSCA guidelines.

g) Assure that identification of all samples is adequate to ensure no confusion regarding sample identity during testing.

h) Perform all seed health test in accordance with the ASTA Ad Hoc Watermelon Fruit Blotch Committee.

i) Maintain test records, documents and any other pertinent test information for a period that correspond with at least one year beyond the inventory life of the product.

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